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Project Title: **Variation in molecular pathotypes of *Escherichia coli* O26 recovered from bovine feces in the United States.**

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Objective: To characterize the distribution of molecularly defined *Escherichia coli* O26 pathotypes within bovine feces.

Methods: *Escherichia coli* O26 strains (n=179) isolated from U.S. feedlot cattle were investigated in this study. DNA was extracted from these *E. coli* O26 isolates and sent to The French Agency for Food, Environmental and Occupational Health (ANSES). High through-put real-time PCR was used to investigate these isolates against twenty-five published markers including, but not limited to: O26 serogroup-specific markers and virulence associated markers. Genetic marker combinations allowed for these isolates to then be classified into pathotypes — EHEC, EHEC-like, and EPEC. Enterohemorrhagic *E. coli* (EHEC) are positive for stx_1 and/or stx_2, *eae*, *espK*, and can be associated with virulence factors. The absence of the Shiga-toxin genes (stx_1 and stx_2) differentiates the EHEC-like pathotype from EHEC. Enteropathogenic *E. coli*

(EPEC) are defined as *eae* positive and negative for *stx* and virulence factors. EPECs have further been described as typical and atypical (AEEC); the latter not associated with hemolytic uremic syndrome (HUS) or the bundle-forming pilus *bfpA*.

Key Results: Selected markers were capable of distinguishing these O26 isolates into molecularly-defined pathotypes, with a large proportion of isolates classified as AEEC. However, within an individual pathotype a low genetic diversity was observed. To further discern this genetic diversity, the AEEC isolates are undergoing analysis of their clustered regularly interspaced short palindromic repeat (CRISPR) loci. CRISPRs are currently being used as a subtyping method for *Salmonella* and the intrinsic locus diversity has previously been characterized for different bacteria genera. Our preliminary data build upon the development of CRISPR typing as a more efficient and definitive method for establishing cases and sources of foodborne outbreaks associated with products of bovine origin.

Application to the Industry: *E. coli* O26 have been identified as the most common non-O157 STEC to cause human illnesses in the U.S. and has been implicated in outbreaks around the world. *E. coli* has a high genomic plasticity, virulence factors on potentially mobile elements (phage, plasmids, and pathogenicity islands), which allow it to readily lose or acquire virulence factors. Current testing methods for identifying adulterated products lack validity in distinguishing pathotypes belonging to the O26 serogroup, which can lead to negative implications in the food industry through misidentification or absence of identifying a pathotype. The investigation of specific virulence factors provided in this research help to further understand the diversity of *E. coli* O26 pathotypes in bovine feces.